# Decreased Skin Superoxide Dismutase Activity by a Single Exposure of Ultraviolet Radiation Is Reduced by Liposomal Superoxide Dismutase Pretreatment

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The effect of a single exposure to UV radiation on skin superoxide dismutase (SOD) activity was examined in mice. A significant decrease in SOD activity was observed 24 and 48 h after UV irradiation, returning to the normal level by 72 h after irradiation. Decreased SOD activity after UV exposure was reduced by pretreatment with liposomal SOD (L-SOD). This protective effect of L-SOD may have potential clinical application for photodermatologic reactions.
*J Invest Dermatol 89:111–112, 1987*

Since the skin is always in contact with oxygen and is occasionally exposed to ultraviolet (UV) light in the presence of surface lipid, photooxidative damage induced by photoreactive oxygen species inevitably occurs [1]. Recently, photodermatologists have become increasingly interested in the role of reactive oxygen species (ROS), and several photodermatologic reactions, including not only porphyrias and phototoxic reactions but also sunburn cell formation [2] and contact photosensitivity [3], have been shown to be related to ROS. Superoxide dismutase (SOD), which catalyzes the reduction of superoxide anion ($O_2^-$), serves to protect the skin from photooxidative tissue injury. When the production of ROS is beyond the capacity of available SOD, photooxidative damage occurs.

In this study, we have investigated the effect of a single exposure to UV radiation on the skin SOD activity. We have also evaluated whether systemic pretreatment with liposomal-SOD (L-SOD) reduces the UV-induced decrease in skin SOD activity.

## MATERIALS AND METHODS

**Animals** Male BALB/c mice were obtained from Shizuoka Experimental Animal Corporation, Hamamatsu, Japan, and used in all experiments when they were 8–12 weeks old.

**Exposure to UVB** Sun lamps (FL20SE) emitting UVB ranging from 280–320 nm with a peak emission at 305 nm were purchased from Toshiba Electric Co., Tokyo, Japan. As measured by UV radiometer (Tokyo Kogaku, Tokyo, Japan), the energy output of the 3 sun lamps at a distance of 20 cm was 2.1 mW/cm$^2$ at 305 nm and less than 0.01 mW/cm$^2$ at 365 nm.

The abdominal wall skin of the mice was shaved and the site was irradiated with the 3 sun lamps at a dose of 1 J/cm$^2$. The mice were sacrificed at 3, 24, 48, or 72 h after UVB treatment and the skin samples were obtained for SOD activity assays.

**Liposomal-SOD Treatment** The L-SOD and empty liposomes were the generous gifts of Dr. A. M. Michelson, Institute de Biologie Physico-Chimique, Paris, France. The L-SOD encapsulated bovine Cu- and Zn-SOD facilitates fixation and penetration of the enzyme as a function of liposome composition, thus increasing the physiologic lifetime [4–6]. The empty liposomes without SOD were prepared by Dr. A. M. Michelson. The L-SOD and empty liposomes were dissolved in sterile physiologic saline. Each mouse was injected i.p. with a solution (0.2 ml) containing 100 $\mu$g of L-SOD or an equal amount of empty liposomes 2 h before exposure to UVB radiation. Each experimental group consisted of 3 mice.

**Skin SOD Activity Assay** Tissue homogenates including both dermis and epidermis were prepared from the skin biopsy specimens in 125 mM phosphate buffer using a Teflon homogenizer.

The SOD activity in the skin was assayed by applying our method previously described for blood cells [7,8]: 5% skin homogenates obtained as described above were diluted to 0.08% in Triton X-100, kept on ice for 1 h, and centrifuged for 10 min at 7000 *g*; 0.5 ml of the supernatant was added to 2 ml of an assay mixture of our xanthine-xanthine oxidase $O_2^-$ generating system. In this system, the formation of $O_2^-$ was determined by ferricytochrome c (type III) reduction, and the absorbance was measured at 550 nm by spectrophotometer (Beckman, UV5260, U.S.A.). The amount of SOD in the sample sufficient to inhibit the rate of reduction of cytochrome c by 50% was defined as 1 unit of activity, and expressed as unit/mg protein.

In the SOD activity assay of the skin specimens, the samples not only inhibited cytochrome c reduction but also directly reduced a small amount of cytochrome c without the mediation of $O_2^-$. Therefore, the actual value of SOD activity induced by the skin homogenates was calculated by taking the amount of directly reduced cytochrome c into consideration; the actual SOD activity was obtained from the formula:

$$\text{Unit} = \frac{a - (b - c)}{\frac{a}{2}}$$

Manuscript received October 31, 1986; accepted for publication December 31, 1986.

This work was supported in part by grants from the Lydia O'Leary Memorial Foundation and the Japanese Dermatologists Association.

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Abbreviations:

L-SOD: liposomal superoxide dismutase
ROS: reactive oxygen species
SOD: superoxide dismutase
UV: ultraviolet

0022-202X/87/$03.50 

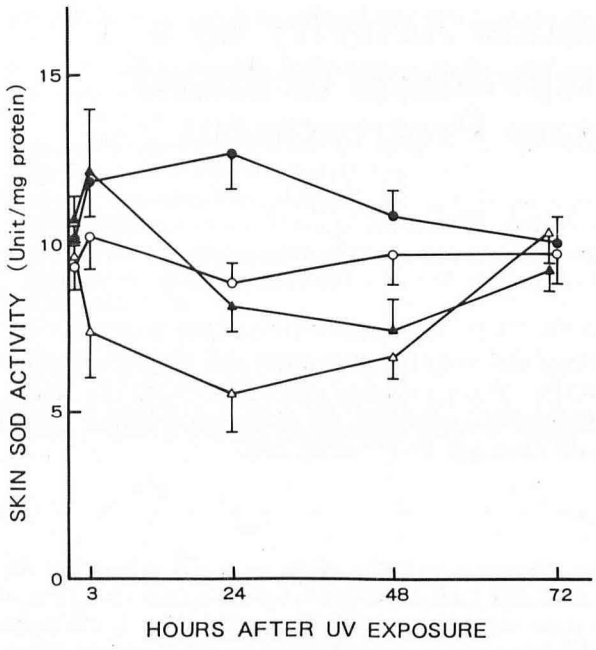


**Figure 1.** The effect of UVB irradiation on skin SOD activity with or without L-SOD pretreatment in mice. The skin specimens were obtained 3, 24, 48, and 72 h after exposure to UVB (0 or 1 J/cm$^2$) with or without L-SOD pretreatment (100 μg/mouse). Empty liposomes alone (control) (*open circles*), L-SOD (100 μg) alone (*solid circles*), empty liposomes and UVB exposure (1 J/cm$^2$) (*open triangles*), L-SOD (100 μg) and UVB exposure (1 J/cm$^2$) (*solid triangles*).

where a is absorbance obtained by the addition of xanthine oxidase alone, b is absorbance obtained by skin homogenates in the presence of xanthine oxidase, and c is absorbance obtained by the addition of skin homogenates alone before the addition of xanthine oxidase.

Since the percent inhibition did not parallel the real SOD activity above, approximately 1 unit, it was adjusted according to the formula of Asada et al [9].

**Statistical Analysis** Student's *t*-test was employed to determine the statistical significance of the differences between means.

## RESULTS

As shown in Fig 1, a single UVB irradiation significantly decreased the skin SOD activity at 24 and 48 h after exposure when compared with the control ($p < 0.01$). This reduction in SOD activity was insignificant at 3 h and seemed to have recovered by 72 h. Significantly increased SOD activity was observed at 24 h in the L-SOD treated group when compared with the appropriate control ($p < 0.01$). Of great interest is the finding that the decrease in skin SOD activity produced by UVB exposure was reduced at 3 ($p < 0.01$) and 24 h ($p < 0.05$) by L-SOD pretreatment.

## DISCUSSION

The present study demonstrates that a single exposure to UVB radiation significantly decreases the skin SOD activity, but this effect is reduced by L-SOD pretreatment. The protective role of skin SOD against toxic ROS is an important factor that modulates cutaneous oxidative damage [10]. Because the skin is directly exposed to UV radiation and to substances known to generate ROS in the presence of oxygen, these photoreactive oxidants and the skin SOD activity are the major components that modify photobiologic cutaneous reactions. Although chronic exposure to UV radiation elevates the skin SOD levels [11], exogeneously given SOD might be even more helpful to keep the skin safe from photooxidative tissue injuries. Therefore, the protective effect of L-SOD administered either topically or systemically may have potential clinical application for the prevention of photodermatologic reactions, aging, or even carcinogenesis.

*We are very grateful to Dr. A. M. Michelson for giving us the opportunity to use L-SOD.*

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